

SOME METHODS OF HISTOLOGIC TECHNIQUE.*

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In carrying on original work and in obtaining good results in the course in normal histology in the laboratory under our charge, it has been a problem of considerable interest to reduce to the smallest possible amount the time and energy expended in the work of preparing material for teaching a class averaging about sixty students. While it is impossible to lay down any fixed rule in work of this character to be the guide of the student in making preparations for the study of normal histology, we believe that by the employment of simple methods which will meet the largest number of requirements in preparing tissues for examination, and recommending these to the student, we reap the greatest benefit from the necessarily limited amount of time allotted to such a course. While points specially alluded to in this paper are not original entirely, but are the result of long observation of the working laboratory courses of various teachers, I am satisfied that quite a number insure accuracy in work with the least possible expenditure of time and attention.

The student is taught from the beginning the general principles of post-mortem examination, cautioned as how to be selective in material for normal and pathological histology, taught the various methods of staining technique, and preparation of slides for his individual study. To ensure the most accurate results, so far as the relationship of tissues are

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concerned, as well as for their immediate preservation, formalin has been used as a hardening and fixing agent. From the time the selected material starts upon its course of preparation it is designated numerically, and the name of the object or specimen immediately entered in a register, which I shall describe later. A great waste of time and the loss of many valuable specimens result from the placing in the single preservative of specimens which were not previously marked for identification. The method employed now, and which has worked favorably, is to tag each preparation with a specially prepared pure tin tag. The tagging material may be procured in sheet form and of very thin quality, and tags of any size desired by the preparer can be made by simply marking off the dimensions upon the sheet. It is my custom to prepare, say, 400 tags at a time, and number them in serial order by means of small dies and afterward cutting the sheet into strips. The most convenient size I have found to be one inch by three-sixteenths. When cut of this size they take up very little room in the smallest specimen jars, allowing of many preparations to be placed in a single bottle. With a small punch, at the right hand side of the tag, a hole is made, through which a strong, but delicate, linen thread can be attached for the purpose of fastening the tag to the specimen. It is found most convenient to fasten these to the specimen by allowing, say, only $1\frac{1}{2}$ inch of the string to project. If they are made longer, and many preparations are put together in a jar, they are apt to become knotted and considerable delay is caused in separating preparations while passing them from one reagent to another. These tags have several advantages: they are perfectly preserved in any of the common preservative fluids without tarnishing, corroding, or in any way obscuring the number which has been stamped upon them; if dies are not handy, the number or even the name of the preparation can easily be scratched upon the tag with the point of a sharp instrument; they can be used repeatedly if it is a question of economy in the purchase of the material, although it is extremely cheap and if one is

conducting considerable work it is more satisfactory to keep up a consecutive system of numbering. I prefer the numerical registering of preparations for the following reason: There is no possibility of duplication, and furthermore, with the numbers properly described in the alphabetical register prepared for the purpose, the preparation is known from the beginning to the end by its number, and no possible confusion can result.

The material secured for future microscopic preparation is immediately tagged in the manner before described, and from that time goes forward through the various fixing, hardening, embedding and staining reagents with the tag affixed. If properly attached to the most delicate anatomical preparation they are not likely to get away from it, if ordinary care is used; in dense tissues it is almost impossible, without destroying the tissue, to separate them. It is my custom, when preparing a number of fresh tissues, to give them their respective serial numbers, carrying at the same time through the various reagents until the point of embedding is reached, when a further and special use of the tag presents itself. I formerly embedded all preparations in the more common forms of water bath. So far as the embedding process was concerned, it was as perfect as the method I now use, but it had the disadvantage of always having the paraffin in metal cups, which made it impossible, without exceptional conditions of light, to define one specimen from another. I now employ ordinary glass cylinders, commonly made for staining jars, and place my specimens in the paraffin in a hot-air bath governed in the regular way with a thermostat. After the allotted time for proper infiltration, one can open the door of the bath and readily see any preparation through the clear melted paraffin in the glass cylinder. As it is of importance just at this stage of preparing, I should have stated that it is particularly necessary to attach the tag to the specimen in such a way as to indicate that the preparation should be placed in the imbedding block in a particular position. It takes but a moment to do this at the time of cutting

out material, and saves time, which is of great importance, at the actual moment of embedding, when, if the process is delayed too long, the saturated specimen stiffens and the surrounding paraffin in the mold is not of the same density. The special advantage of the tagging system at this point, that is, in transferring the specimen from the bath to the cutting block, is that you can seize the tag with your forceps, withdraw the specimen from the melted paraffin and instantly transfer it into the mold which is to receive it, and which has previously been filled with melted paraffin. For these molds I have used for a number of years small sections of lead pipe, which I keep in stock of four or five different diameters, also of several different sizes in lengths from $\frac{3}{4}$ inch to 2 inches. The advantages of molds of this kind are that they are sufficiently thick to be firm on the base, when touched with a little melted paraffin and placed upon an embedding slab they are firmly seated and prevent the melted paraffin from running out at the edge, and furthermore, when the block becomes hard with the enclosed preparation, the specimen is without any trouble or unnecessary pressure removed from the mold. The specimen is transferred from the bath to the embedding mold, and when placed in the desired position for cutting, the metal tag is allowed to drop over the edge of the mold. As will be readily seen, if you remove either a few or a large number of embedded blocks from these molds, the firmly-attached tag serves to identify the preparation at any time. As there is always an excess of paraffin about a piece of tissue, I immediately trim away until the block is in a suitable shape for the jaw of the microtome, taking care not to cut the attached string, and after it is properly prepared I gently heat the bottom of the paraffin and press my metal tag directly onto it. This keeps the number conveniently attached to the block for future use.

Another point of great convenience I have found to be in the employment of carton boxes, of the character and size accompanying this paper. It was formerly my custom, in

the care of a large number of preparations, which were accumulated from years of hospital and laboratory work, to wrap the paraffin blocks in special paper wrappers and number and label these; they were then kept in compartments accommodating fifty each, in numerical order. As few preparations were of uniform size, the collection of blocks for ready reference in this way was far from satisfactory. As soon as a block has been prepared for the microtome it is immediately placed in one of these carton boxes, which is numbered to correspond with the preparation; these boxes being uniform in size, and adapted to the largest sized preparations commonly used, are most conveniently kept in a cabinet containing shallow drawers which will hold 200 boxes each, one layer deep. The cabinet I use consists of six such drawers, will hold 1,200 blocks arranged in this way, all in numerical order, and turning from the index to the cabinet, any desired block can be secured at once, and of course is readily returned to its place.

Concerning the matter of indexing one's material in a large laboratory, I find the following plan to work to my entire satisfaction, having had it in practice for about five years, and it has met all my requirements. I divide my material, as the work in my laboratory has been duly divided in the normal and pathological instruction, into these two respective classes—Normal and Pathological. Taking several of my most comprehensive works treating of each of these subjects, I analysed the indices and prepared an index of my own, which was the result of getting together the essential points contained in these text-books. Using an index book, I then placed in it, in strict dictionary order, the essential subjects which I expected to collect in normal histology and in pathology. In my register proper, contained in the same book, I devote one-half of it to the normal subject and the other half to the pathological. The material that I had at that time, which was not of small amount, I then entered, allowing a suitable division of space for the title, date, and description, and any memoranda relating to it in its proper place on the

page of my register, where it bears a corresponding number of the block. Under the subject, then, to which this particular preparation belongs, say angioma, for example, I refer to it in my already prepared index simply by number. The index, on the whole, was a very complete one in the beginning for the purpose for which it was intended, and I have added very few titles in the long time it has been in use. It shows at a glance about how much material I have of any subject, refers me to the blocks instantly by number, and shows me in what material or in what subject I am scant, or in which I have nothing at all; in other words, it is a good index of the desiderata, as well as what you have on hand. In my register, as I receive material, I enter the title of it in the order it is received, whether it be normal or pathological. In my index I separate the normal from the pathological, although contained in the same alphabet and upon the same page. I index the normal subjects in black ink and the pathological in red. With an index of titles of considerable size, as this one is, I believe this method reduces to the least possible space the large number of subjects, and still allows ample room for the entering of a great many reference numbers; this serves the double purpose of an index by referring to the proper location of that particular material in the register, as well as to the block, and its location in the cabinet.

Just another point regarding matter of technique, but one which may prove useful to some member of the Society, as it has proved to me. In the last dozen years I have followed through all the various stages of fixing preparations to the slide for staining purposes, and have tried with more or less success about everything that has been recommended for this particular purpose. During the last few years I have used the common gelatin mixture which is recommended by Dr. Piersol and Dr. Gray, but found that in certain methods, while it had many advantages, it had its disadvantages also. The most trouble was experienced in staining with the hematoxylin stain and contre stain. I found when it was

used according to the formula first recommended, namely about half per cent., that it was not uncommon for the extremely delicate gelatin film to take up a small amount of the stain, especially about the border of the preparation. I used it even as dilute as one-tenth per cent., and yet, in some cases, with the same troublesome result. Every effort and much time was spent in freeing the slide from the excess of the gelatin mixture, but, nevertheless, there was always a small amount of it perceptible.

I am indebted to Dr. Walter Reed, U. S. A., for the suggestion of the method which I now employ, and have used with perfect success. Knowing that he was using similar methods of staining, I asked his experience as regards a fixative. He informed me that he had, in some cases, similar trouble, and told me that he was using only distilled water for the purpose of fixing sections on the slide. I followed his suggestion, and since that time, now nine months, it has been extremely rare for the preparation to become detached from the slide. I will say that, as an illustration, I cut and fastened to the slide, by this method (simply placing the section upon the distilled water), 1,640 individual sections. These were afterwards carried through the usual process of staining, cleaning, etc., and without the loss of a single section. While the method is in the main commonly understood, it may not be out of place to say, in a few words, how time and labor is saved in this part of the work. It is commonly my custom to prepare twenty-four or more slides at a time of a particular object; I place these in a tray holding two dozen, which is commonly employed with the ordinary drying bath, the slides depending upon the size of the section, are covered with distilled water, and the sections cut and placed upon the water. The entire set is then placed in my drying oven sufficiently long, and at a proper temperature, to smooth the sections entirely, taking care that the temperature never reaches the melting point of the paraffin. The tray of slides is then removed, each section accurately centered in the desired position on the slide, the excess of water allowed

to run off, and the slide placed upon end in a rack for drying. The racks that I employ, while not original in any sense, are well adapted to this special part of the work, and I submit one for examination. They are made to hold seventy-two slides, a very convenient size, and of a material which will not warp and allow of an irregular arrangement of the slides.